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Genetics of multiple disease resistance in a doubled-haploid population of barley

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With 3 tables

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Abstract

The barley accession Q21861 possesses resistance to the stem-rust (*Puccinia graminis* f.sp. *tritici*), leaf-rust (*P. hordei*), and powdery-mildew (*Blumeria graminis* f.sp. *hordei*) pathogens. An anther-culture-derived doubled-haploid population was produced from F_1 plants from a cross of this accession and the susceptible breeding line SM89010 as a means of rapidly and efficiently determining the genetics of multiple disease resistance. The doubled-haploid population segregated 1:1 (resistant : susceptible) for resistance to the stem rust pathotype QCC indicating the involvement of a single resistance gene, *rpg4*. Two-gene (3:1) and one-gene (1:1) segregation ratios were observed for resistance to the stem-rust pathotype MCC at low (23–25°C) and high (27–29°C) temperature, respectively. These different segregation patterns were due to a pathotype × temperature interaction exhibited by *rpg4* and *Rpg1*, another stem-rust-resistance gene present in Q21861. One-gene and two-gene segregation ratios were observed in reaction to the leaf rust and powdery mildew pathogens. These data demonstrate the utility of doubled haploid populations for determining the genetics of multiple disease resistance in barley.

Key words: *Hordeum vulgare* — *Puccinia graminis* f.sp. *tritici* — *Puccinia hordei* — *Blumeria graminis* f.sp. *hordei* — multiple disease resistance — doubled haploids — genetic analysis

The use of doubled haploids in barley (*Hordeum vulgare* L.) breeding and genetic studies has increased markedly in recent years. For breeding, the production of doubled-haploid lines (DHLs) offers one of the fastest ways of obtaining completely homozygous progeny from selected crosses. Additionally, since all the alleles of DHLs are fixed, selection for quantitative characters is often more reliable than in conventional barley populations (Choo et al. 1985). The utility of DHLs in breeding programmes has been demonstrated, as a number of barley cultivars have been developed using this system (Pickering and Devaux 1992). DHLs have also proven valuable in genetic studies on linkage relationships (Pickering and Devaux 1992), quantitative inheritance (Choo et al. 1985), pleiotropy (Kjær et al. 1990), and molecular mapping of the barley genome (Graner et al. 1991, Heun et al. 1991, Kleinhofs et al. 1993). Little attention has, however, been directed toward the use of doubled haploids for studying the genetics of multiple disease resistance in barley. Doubled-haploid populations are ideal for this type of research because assessments for the infection phenotype of individual DHLs can be made with several pathogens in simultaneous inoculation experiments (or in experiments conducted sequentially in time) and as often as necessary to obtain unequivocal readings.

In the northern Great Plains of North America, stem rust (causal organism: *Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. & E. Henn.) has historically been one of most important diseases of barley. However, since 1942, this disease has been controlled through the wide use of the resistance gene *Rpg1* in commercial cultivars (Steffenson 1992). The effectiveness of the *Rpg1* resistance was recently compromised by the appearance of a virulent stem-rust pathotype (Pgt-QCC) in 1989. This outbreak of stem rust prompted the initiation of a programme to identify resistance to this new virulence type in a diverse collection of barley germplasm (Steffenson 1992). From these germplasm evaluations, the accession Q21861 was identified as possessing one of the highest levels of resistance to pathotype QCC at both the seedling and adult plant stages of growth (Jin et al. 1994a). In addition to possessing resistance to pathotype QCC, Q21861 also carries *Rpg*1 and genes for resistance to the leaf rust (*Puccinia hordei* G. Otth) and powdery mildew (*Blumeria graminis* DC. f.sp. *hordei* Ém. Marchal) pathogens (Steffenson et al. 1992). Information regarding the inheritance of resistance to these pathogens in Q21861 will facilitate the selection and transfer of resistance genes into advanced breeding lines. In this study, we report on the use of doubled haploids as a rapid and efficient means of determining the genetics of multiple disease resistance in barley.

Materials and Methods

Plant materials: A total of 129 DHLs were produced by anther culture (Kao 1981, Kao et al. 1991) from F_1 plants of the cross Q21861 × SM89010. Q21861 is an accession of unknown parentage that was originally selected in 1983 from a barley breeding nursery established at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. SM89010 is a two-rowed malting barley line from the University of Saskatchewan breeding programme. It was derived from the cross Nairn × Manley and is susceptible to stem rust, leaf rust, and powdery mildew. To induce call as formation on microspores from F_1 plants, the M40 medium of Kao et al. (1991) was used with the following modifications: glucose and sucrose were omitted; 2 mg of 2,4-D (2,4-dichlorophenoxyacetic acid) were replaced by 0.5 mg of NAA (napthyl acetic acid); and the amount of Ficoll (Pharmacia) and maltose added was 200 and 60 g, 300 and 60 g, or 200 and 90 g, respectively (Hunter et al. 1988, K. N. Kao, unpubl. data).

Pathogen cultures: Single uredinial cultures of the *P. graminis* f.sp. *tritici* pathotypes Pgt-QCC (culture QCC-2) and Pgt-MCC (culture A-5) were used in this study. On the differential wheat genotypes of Roelfs and Martens (1988), pathotype QCC exhibits an avirulence/virulence formula of *Sr9e*, *Sr7b*, *Sr11*, *Sr6*, *Sr8a*, *Sr36*, *Sr9b*, *Sr30*/*Sr5*, *Sr21*,

Sr9g, *Sr17*, while that of MCC is *Sr21*, *Sr9e*, *Sr11*, *Sr6*, *Sr8a*, *Sr36*, *Sr9b*, *Sr30/Sr5*, *Sr7b*, *Sr9g*, *Sr17*. Pathotype MCC was included in the evaluations because it has proven effective for detecting *Rpg*1 in barley genotypes at the seedling stage (Steffenson et al. 1993b). Leaf rust evaluations were made with the single uredinial culture ND8702 of *P. hordei* which exhibits an avirulence/virulence formula of *Rph2*, *Rph3*, *Rph5*, *Rph6* + *2*, *Rph7*, *Rph9*, *Rph12/Rph1*, *Rph4*, *Rph8*, *Rph10*, *Rph*11 on the set of differential barley genotypes proposed by Steffenson and Jin (1992). Culture ND8702 is representative of the leaf-rust-virulence types that have predominated for over a decade in the midwest region of the USA (Steffenson et al. 1993a). Urediniospores of the rust pathogens were desiccated, sealed in glass vials, and stored at −80°C until needed for inoculation. For the powdery mildew evaluations, a single pustule isolate ND92GH of *B. graminis* f.sp. *hordei* was used. This isolate possesses a narrow virulence spectrum and is useful for detecting a number of powdery-mildew-resistance genes. The avirulence/virulence formula of this isolate is *Mla1*, *Mla6* + *Mla14*, *Mla7*, *Mla9*, *Mla12*, *Mla13* + *Mla*−(*Ru3*), *Mlk*, *Mlg* + *Ml*−(*CP*), *Mla7* + *Ab*, *mlo/Mla3*, *Ml*−(*La*) on the Pallas differential genotypes described by Kølster et al. (1986). Conidia of isolate ND92GH were increased and maintained on a vegetative dwarf mutant of barley (Falk and Kasha 1982) in a greenhouse until needed for inoculation.

Inoculation and assessment of the infection phenotype: Parents and doubled haploid progeny (five seeds) were sown in plastic cones filled with a peat moss:perlite (3:1 ratio) potting mixture and grown at 22–26°C in a greenhouse where supplemental lighting (530–710 μmol photon/m²/s) was provided for 13 h/day. Fertilizer was applied at planting with water-soluble (N15–P0–K15) and controlled release (N14–P14–K14) formulations (Steffenson et al. 1993b). When the first leaves of plants were fully expanded (7 days after planting), inoculations were made with urediniospores of the rust pathogens or conidia of the powdery mildew pathogen. Inoculations with the rust pathogens were made using a concentration of 6.7 mg urediniospores/ml lightweight mineral oil (Soltrol 170) applied at a rate of approximately 1.5 μl oil per plant. After the oil had evaporated from the leaf surfaces (ca. 5 min), plants were placed in chambers maintained near saturation by intermittent mistings (32 s of mist every 16 min) from ultrasonic humidifiers. Plants were incubated in the mist chambers for 16 h at 20–21°C in the dark, exposed to light (120–160 μmol photon/m²/s) as the temperature increased from 21 to 24°C (light treatment given only to plants inoculated with *P. graminis* f.sp. *tritici* (Rowell 1984)), and allowed to dry slowly for approximately 4 h before being placed in one of three environments.

In a recent genetic study involving F_2 populations, a single recessive gene (designated as *rpg4*) was found to confer resistance to pathotype QCC in Q21861 (Jin et al. 1994b). This gene also confers resistance to MCC-a pathotype that has proven effective in differentiating genotypes with *Rpg1* from those carrying all other previously described stem-rust-resistance genes (*Rpg2*, *Rpg3* and *rpgBH*) in barley (Steffenson et al. 1993b). The resistance gene *rpg4* is also temperature sensitive (Jin et al. 1994b); it is effective against pathotypes MCC and QCC at low incubation temperatures (17–25°C) and ineffective at high incubation temperatures (>27°C). Using the two stem-rust pathotypes and different temperature regimes (17–25 or 27–29°C) for incubation, it was possible to determine the putative genotypes of individual DHLs to *P. graminis* f.sp. *tritici* under a hypothesized two-gene model because the resistance genes exhibited a pathotype × temperature interaction. For example, DHLs carrying only *rpg4* would give low-infection types (resistant reactions) to pathotypes QCC and MCC at low incubation temperatures and high-infection types (susceptible reactions) at high temperatures; those carrying only *Rpg*1 would give high-infection types to QCC and low-infection types to MCC under both temperature regimes; and those carrying both *rpg4* and *Rpg1* would give low- and high-infection types to QCC at low and high temperature, respectively, and low-infection types to MCC at both temperatures (Table 1). DHLs lacking both of the resistance genes would give high-infection types at all pathotype × temperature combinations. In this investigation, three of the four possible pathotype × temperature combinations were used to resolve the putative genotypes of the DHLs: 1. Inoculation with pathotype QCC and incubation at 17–22°C where only *rpg4* would be effective; 2. Inoculation with pathotype MCC and incubation at 23–25°C where both *rpg4* and *Rpg*1 would be effective; and 3. Inoculation with pathotype MCC and incubation at 27–29°C where only *Rpg*1 would be effective. Plants inoculated with pathotype QCC were incubated in a greenhouse, and those inoculated with MCC were incubated in a growth chamber with a 13-h photoperiod (120–160 μmol photon m²/s).

Table 1: Postulated infection types of Q21861/SM89010 doubled haploid genotypes infected with pathotypes QCC and MCC of *Puccinia graminis* f. sp. *tritici* and incubated under two temperature regimes. Low and high incubation temperatures are 17–25°C and 27–29°C, respectively. Low infection types (LITs) include 0, 0;, 1, and 2, whereas high infection types (HITs) include 3 and 4. Data are based on results from Jin et al. (1994b)

	Pathotype			
	QCC		MCC	
Host genotype	Low temp.	High temp.	Low temp.	High temp.
rpg4rpg4/rpg1rpg1[1]	LIT	HIT	LIT	HIT
Rpg4Rpg4/Rpg1Rpg1	HIT	HIT	LIT	LIT
rpg4rpg4/Rpg1Rpg1	LIT	HIT	LIT	LIT
Rpg4Rpg4/rpg1rpg1	HIT	HIT	HIT	HIT

[1] *rpg4* is the allele in Q21861 that confers resistance to pathotypes QCC and MCC at low incubation temperatures. *Rpg*1 is another allele at a different locus in Q21861 that confers resistance to pathotype MCC at both low and high incubation temperatures

For the leaf-rust evaluations, plants were incubated in a greenhouse at 17–22°C. Inoculations with the powdery mildew pathogen were made by shaking conidia from the infected dwarf barley plants onto the parents and DHLs. These plants were then incubated at ambient greenhouse conditions (22–25°C).

At 12–14 days after inoculation, the infection types of plants were assessed using the 0–4 scale for stem rust (Stakman et al. 1962, as modified by Steffenson et al. 1993b for barley), leaf rust (Levine and Cherewick 1952), and powdery mildew (Mains and Dietz 1930). Infection types 0, 0; (in the case of the rusts), 1 and 2 were considered indicative of host resistance (low infection type), whereas infection types 3 and 4 were considered indicative of host susceptibility (high infection type). Experiments with the individual pathogens or pathotypes were conducted in a randomized complete block design with two replicates and were repeated twice. Due to limited growth-chamber space, some of the disease evaluations were made sequentially in time. Segregation ratios were subjected to χ^2 analyses.

Results

The infection types observed on progeny (data not shown) were very similar to those exhibited by the parents (Tables 2, 3) to *P. graminis* f.sp. *tritici*, *P. hordei*, and *B. graminis f. sp. hordei*. Infection types on individual DHLs were consistent within and between experiments and allowed for the unequivocal classification of progeny into resistant and susceptible groups. The Q21861/SM89010 DHLs segregated 1:1 for resistance: susceptibility to pathotype QCC, indicating the involvement of a single gene for resistance (Table 2). The ratio of resistant:susceptible DHLs to pathotype MCC was 3:1 at the low incubation temperature (23–25°C) and 1:1 at the high incubation temperature (27–29°C) (Table 2). Thus, two genes (*Rpg1* and *rpg4*) were effective in conferring resistance to this pathotype at low temperature, and only one gene (*Rpg1*) was effective at high temperature. Based on the pathotype × temperature interaction of these genes, it was possible to determine the putative genotypes of the DHLs for reaction to *P. graminis* f.sp

Table 2: Parental infection types and segregation of Q21861/SM89010 doubled haploid lines to pathotypes QCC and MCC of *Puccinia graminis* f. sp. *tritici* under different incubation temperatures. Progeny exhibiting infection types 0, 0;, 1, or 2 were classified as resistant and those exhibiting infection types 3 and 4 were classified as susceptible. The parental infection types are given as the two most common and the lowest and highest types observed. The superscript symbols + and − denote more or less sporulation of uredinia, respectively

Pathotype (incubation temperature)	Parental infection types Q21861 Most common	Q21861 Low/high	SM89010 Most common	SM89010 Low/high	Segregation ratio resistant : susceptible Observed	Expected	χ^2	Probability
QCC (17–22°C)	0,0;	0/1	3^-,3	$3^-/3^+$	58:71	1:1	1.31	0.252
MCC (23–25°C)	0,0;	0/1	$3,3^-$	$2/3^+$	90:39	3:1	1.88	0.170
MCC (27–29°C)	0,0;	0/2	$3,3^-$	$3^-/3^+$	58:71	1:1	1.31	0.252

tritici. The numbers of DHLs with the putative genotypes *rpg4rpg4/rpg1rpg1*, *Rpg4Rpg4/Rpg1Rpg1*, *rpg4rpg4/Rpg1Rpg1r* and *Rpg4Rpg4/rpg1rpg1* were 32, 32, 26 and 39, respectively. This segregation fit closely with the expected 1:1:1:1 ratio ($\chi^2 = 2.63$; $P = 0.453$) for the genotypic frequency of two unlinked genes.

The DHLs segregated 52 (resistant): 77 (susceptible) to isolate ND8702 of *P. hordei* (Table 3). The best fit for this segregation pattern was with a 1:1 ratio; however, the χ^2 value (4.85) was still significant ($P = 0.028$). To isolate ND92GH of the powdery mildew pathogen, the DHLs segregated 3:1 for resistance : susceptibility (89:40) indicating that two genes were involved in conferring resistance (Table 3).

Discussion

Q21861 is a barley accession that possesses resistance to a number of important pathogens, including pathotype QCC of *P. graminis* f.sp. *tritici*. In this study, the genetics of multiple disease resistance in this accession was rapidly and efficiently determined from a doubled haploid population. Doubled haploid populations offer several advantages over conventional F_2 populations for the analysis of multiple disease resistance. Since DHLs are completely homozygous and 'immortal', evaluations with different pathogens can be made simultaneously or sequentially in time on each individual genotype and in replication as needed. Some of the inoculation experiments in this study were conducted sequentially over time. Had sufficient growth chamber space been available for simultaneous disease tests, the genetics of resistance to stem rust (two different pathotypes), leaf rust, and powdery mildew could have been determined in as few as 19 days. The immortal nature of this population facilitated the easy detection of the two stem-rust-resistance genes under the different pathotype × temperature combinations and also the genes for resistance to *P. hordei* and *B. graminis* f.sp. *hordei*. Although the infection types of DHLs were distinct and reproducible in this study, the ability to repeat disease assessments on such genotypes is important especially when the expression of the phenotype is variable (Melchinger 1990, Heun 1992). Another advantage of using DHLs is that possible linkage relations among genes can be readily resolved. This information can aid in identifying chromosomal locations for genes of interest providing that the position of the other gene(s) is known. In this study, linkage tests were made among all disease resistance genes and with the single gene *S* which controls rachilla hair length and is located on chromosome 7 (Søgaard and von Wettstein-Knowles 1987). The only linkage detected was with the leaf rust resistance gene and *S* ($\chi^2 = 9.51$ with recombination value = 36.4%). This association is, however, considered tenuous because the segregation of progeny with long (78): short (51) rachilla hairs was distorted (for a 1:1 ratio: $\chi^2 = 5.65$ with $P = 0.017$). However, preliminary linkage analyses using molecular markers suggest that the leaf-rust-resistance gene is located near the centromere of chromosome 7 (A. Kilian et al. unpubl. data). The attributes discussed above for doubled-haploid populations could not be easily determined using conventional early generation progeny analyses. Even if two different pathogen reactions could be assessed on a single F_2 plant (e.g. split-leaf inoculations or inoculation of one pathogen on the first leaf and a second on the second leaf), interactions between the pathogens could confound the results. Using the doubled-haploid technique, homozygous lines can be obtained in the shortest possible time, an important factor in breeding programmes (Choo et al. 1985). If a DHL is identified with all the desired genes, it can be used

Table 3: Parental infection types and segregation ratios of Q21861/SM89010 doubled haploid lines to isolate ND8702 of *Puccinia hordei* and isolate ND92GH of *Blumeria* graminis f. sp. *hordei*. Progeny exhibiting infection types 0, 0; (with *P. hordei* only), 1, or 2 were classified as resistant and those exhibiting infection types 3 and 4 were classified as susceptible. The parental infection types are given as the one or two most common and the lowest and highest types observed. The superscript symbol − denotes reduced sporulation of uredinia

Pathogen (isolate)	Parental infection types Q21861 Most common	Q21861 Low/high	SM89010 Most common	SM89010 Low/high	Segregation ratio resistant : susceptible Observed	Expected	χ^2	Probability
Puccinia hordei (ND8702)	0;,1	0;/1	3^-	$3^-/3$	52:77	1:1	4.85	0.028
Blumeria graminis f. sp. *hordei* (ND92GH)	0	0	4	4	89:40	3:1	2.48	0.115

at once as a parent in the next crossing cycle or may even be increased for release as an improved cultivar. From the disease evaluations in this study, four of 129 DHLs carried together *rpg4* and *Rpg1*, the leaf-rust-resistance genes, and at least one of the two powdery-mildew-resistance genes detected. These lines are being evaluated for agronomic performance in the field at several locations and will be released as multiple disease-resistant germplasm.

Doubled haploids are not without their limitations for investigations on the genetics of multiple disease resistance. Firstly, since DHL production requires skilled personnel and the availability of controlled environments (Choo et al. 1985), this technique cannot be routinely used by all projects. Secondly, with doubled haploids, it is not possible to determine whether a gene is dominant or recessive since both alleles are expressed. However, this information could be obtained from F_1 plants prior to the development of DHLs, providing, of course, that the disease evaluation is non-lethal. Thirdly, doubled-haploid populations may not represent an unbiased sample of parental gametes. To accurately determine the number of genes controlling a specific trait, the progeny population must contain a random sample of parental gametes. This assumption has not proven valid in some doubled haploid populations derived from anther culture (Powell et al. 1986, Graner et al. 1991, Thompson et al. 1991). In this investigation, distorted segregation was observed for rachilla hair length and *P. hordei* resistance; this was probably due to the differential transmission of alleles (*S* and the allele for leaf rust susceptibility) from SM89010 during the anther-culture procedure. This result underscores the need to use caution when interpreting the segregation patterns for traits in doubled-haploid populations derived from anther culture.

The results obtained for the inheritance of resistance to pathotypes QCC and MCC of *P. graminis* f.sp. *tritici* are in agreement with a recent study on F_2 progeny derived from Q21861 (Jin et al. 1994b). Q21861 possesses two genes for resistance to stem rust, *Rpg1* and a newly designated gene, *rpg4*. The latter gene is different from *Rpg2*, according to a previous allelism test. It is also distinct from *Rpg3* based on differences in gene expression (recessive vs. dominant gene action and temperature sensitivity) and reaction to different rust cultures (Jin et al. 1994b). The segregation ratio of DHLs to the leaf rust pathogen suggested the involvement of a single resistance gene; however, the χ^2 value was significant. To verify this hypothesis, an F_2 population was generated from a remnant F_1 seed from the cross Q21861/SM89010 and evaluated for reaction to isolate ND8702. The F_2 population segregated 191:70 resistant : susceptible ($\chi^2 = 0.46$ with $P = 0.497$) thereby confirming the single-gene ratio found with the doubled-haploid population. The identity of the leaf-rust-resistance gene in Q21861 is not known, but is under investigation. Two independently segregating genes were found to confer resistance to isolate ND92GH of *B. graminis* f.sp. *hordei* in Q21861, however, like the leaf-rust-resistance gene, their relation with other reported resistance genes is unknown.

The transfer of multiple disease resistance from Q21861 into agronomically advanced germplasm will help to reduce the potential impact of stem rust, leaf rust, and powdery mildew on barley in the future. Selection of progeny with these resistances can be simplified and made more reliable by molecular tagging of the resistance genes. Toward this end, bulked segregant analysis is being used (Michelmore et al. 1991) to identify RAPD markers that are closely linked to the genes conferring resistance to *P. graminis*. f.sp. *tritici*, *P. hordei*, and *B. graminis* f.sp. *hordei* in the Q21861/SM89010 population.

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